Exhibit D
Boulianne
May 91

THE PRODUCTION OF CHIMERIC MOUSE/HUMAN ANTIBODIES.

G.L. Boulianne,[1,2] N. Hozumi,[1,3] C. Heusser,[4] and M.J. Shulman[1,2]; Department of Medical Biophysics, University of Toronto,[1] and Rheumatic Disease Unit, Wellesley Hospital, Toronto, Ontario,[2] Canada; Ontario Cancer Institute, Toronto, Ontario, Canada[3]; Immunobiologie, Pharmaceutical Research, Ciba-Geigy, Basel, Switzerland CH-4002[4].

Poster #25

Many investigators have proposed that monoclonal antibodies could be useful for the treatment of human disease. Treatment to date has made use of murine monoclonal antibodies where immunization of mice has permitted the generation of antibody producing cells of the desired specificity. It is expected that human antibodies might function better than mouse antibodies in human therapy. However, the use of human monoclonal antibodies of predefined specificity has been limited by the lack of antigen-specific human lymphoid cells. Recombinant DNA techniques raise the possibility of using chimeric immunoglobulin genes composed of human constant regions and mouse variable regions. For this purpose, we have established a model system whereby chimeric genes consisting of mouse variable regions specific for the hapten 2,4,6-trinitrophenyl (TNP) are joined to human constant region genes. When such a gene is transferred to an appropriate mutant hybridoma cell, functional TNP specific antibody production is restored.

GLYCOSYLATION REQUIREMENT FOR BUTYRATE-MEDIATED INDUCTION OF CHORIONIC GONADOTROPIN IN HELA CELLS.

G. Stanley Cox and Daniel S. McClure, Department of Biochemistry, University of Nebraska Medical Center, Omaha, NE 68105.

Poster #26

Accumulation of the glycoprotein hormone α subunit in HeLa cell cultures is enhanced several fold by mM concentrations of sodium butyrate (Btr). This report demonstrates that increased subunit accumulation in response to Btr is dependent on the presence of glucose (Glc) in the growth medium and is inhibited by glycosylation inhibitors such as tunicamycin and 2-deoxyglucose. In contrast, basal levels of subunit synthesis are only marginally affected under similar conditions. The hexoses which support induction of HeLa α in pyruvate medium (glucose > mannose > galactose > fructose) are identical to those which have been shown previously to stimulate the glucosylation of lipid-linked oligosaccharides. Glc and Btr cause a decrease in [^{3}H]mannose-labeled cellular glycoproteins and a concomitant increase in the fraction of label recovered in secreted glycoproteins. Likewise, Glc stimulates the incorporation of [^{3}H]glucosamine into immunoprecipitable α subunit relative to the bulk of HeLa secreted glycoproteins, and this is further enhanced by Btr. Lectin chromatography of conditioned media demonstrates that a nonglycosylated subunit does not accumulate in pyruvate medium, either in the absence or presence of Btr. Inhibition of butyrate-mediated induction by deoxyglucose can be partially overcome by the addition of protease inhibitors to the culture medium, suggesting an effect of protein glycosylation on subunit turnover. Taken together, these results demonstrate preferential accumulation of glycosylated hormone subunit in response to Glc and Btr and suggest that expression by tumors of some ectopic proteins may be regulated at the post-translational level.